Effect of High versus Low Oral Doses of Valacyclovir on Herpes Simplex Virus-1 DNA Shedding into Tears of Latently Infected Rabbits

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Purpose. To assess the effect of high doses of valacyclovir (VCV) on HSV-1 DNA shedding into tears of latently infected rabbits.

Methods. Three oral doses of VCV were tested. Corneas were inoculated with HSV-1, and latent infection was allowed to establish. Starting on postinoculation (PI) day 28, tear swabs were collected once daily for 6 consecutive days before treatment. The rabbits were placed in five balanced groups: group 1 had no treatment, group 2 received placebo, group 3 received 7 mg/kg VCV, group 4 received 70 mg/kg, and group 5 received 140 mg/kg. The treatment was administered by oral gavage twice daily, starting on PI day 36 and continuing for 14 days. The ocular swabs were collected beginning on PI day 40 and continuing for 10 days.

Results. The mean copy number of HSV-1 DNA before treatment was 370 ± 70, 569 ± 273, 368 ± 86, 408 ± 108, and 396 ± 91, and the mean HSV-1 DNA copy number after treatment was 232 ± 183, 564 ± 186, 518 ± 122, 67 ± 63, and 13 ± 7 in groups 1 to 5, respectively.

Conclusions. There was no observable toxicity in any group. The 70- and 140-mg/kg doses of VCV significantly reduced the HSV-1 DNA copy number, compared with that of the other three groups. A daily dose of 500 mg (~7 mg/kg) VCV in healthy human volunteers did not suppress HSV-1 DNA shedding in tears and saliva. Thus, higher doses of VCV may be necessary to reduce asymptomatic shedding in healthy human subjects. (*Invest Ophthalmol Vis Sci.* 2010;51:4703–4706) DOI:10.1167/iovs.09-4884

HSV-1 is a common cause of loss of vision due to infectious agents in the United States.[1–4] We have shown that more than 90% of the human population have HSV-1 DNA in their trigeminal ganglia (TG) and asymptomatically shed DNA into their tears and saliva.[5–7] Asymptomatic shedding of HSV DNA is a well-defined phenomenon and is a likely source of disease transmission. HSV-1 is easily transmitted through direct contact with lesions and body fluids from latently infected individuals.[1–4,8] Thus, suppression of asymptomatic shedding is one way to reduce or block transmission.

Recently, we tested the effectiveness of a routinely prescribed dose of valacyclovir (VCV) in reducing the asymptomatic shedding of HSV-1 DNA in normal healthy subjects.[7] We found that 500 mg of VCV given once daily did not reduce the asymptomatic shedding of HSV-1 DNA into the tears or saliva. This study was the first in which any dose of VCV was evaluated for the suppression of HSV-1 DNA shedding into tears.[7] A similar dose of VCV has been shown to reduce HSV-2 shedding in immunocompetent subjects with genital herpes.[9] These data may help explain why antiviral drugs have had much less prophylactic efficacy against HSV-1 lesions, including ocular herpes, than against genital herpes, warranting more studies of treatments to inhibit HSV-1 DNA shedding.

Possible strategies to reduce HSV-1 DNA shedding could be the use of a higher dose, a long-term treatment plan, or both. These strategies can lead to adverse side effects caused by VCV. A VCV dose of 2 g four times a day (8 g/d) has been reported to cause mild adverse effects in immunocompetent subjects.[10,11] Lower doses such as 2 to 3 g/d have been used in many studies to treat varicella zoster and HSV-2 subjects.[12–14] Quan et al.[15] used 1 g of VCV three times a day (3 g/d) and had some success (~50%) in the treatment of postherpetic neuralgia. Our hypothesis for this study is to determine whether the higher doses (either 10 times or 20 times the 7 mg/kg dose) of VCV could suppress asymptomatic shedding of HSV-1 DNA into tears of rabbits with latent HSV-1 infection. We tested three doses of VCV to reduce HSV-1 shedding in tears of latently infected rabbits. This study could result in the determination of a dose of VCV that would suppress asymptomatic HSV-1 DNA shedding in human tears.

Materials and Methods

Animals, Cells, Virus, and Drug

All experimental procedures were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the LSU Health Sciences Center

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TABLE 1. Number of HSV-1–Positive Rabbits, Eyes, and Swabs and Mean Copy Number before Treatment, 28 to 33 Days PI

Groups	Positive Rabbits	Positive Eyes	Positive Swabs (%)	Mean Copy Number ± SEM
Untreated	5/5	7/10	12/60 (19)	370 ± 70
Placebo	5/5	5/10	19/60 (31)	569 ± 237
7 mg/kg VCV	5/5	7/10	15/60 (25)	368 ± 86
70 mg/kg VCV	5/5	6/10	15/60 (25)	408 ± 108
140 mg/kg VCV	5/5	6/10	21/60 (33)	396 ± 91

(LSUHSC) Institutional Animal Care and Use Committee. New Zealand White (NZW) rabbits were obtained from McNeil Rabbitry (Moss Point, MS). The McKrae strain of HSV-1 was used to infect the corneas of these rabbits. Before inoculation, the number of viral plaque-forming units (PFU) was determined with a standard plaques assay procedure with CV-1 cells. VCV (Valtrex; GlaxoSmithKline, Research Triangle Park, NC) caplets were used for the oral treatment of the rabbits. The caplets were powdered with a mortar and pestle and suspended in a 1:1 mixture of sweet syrup and suspending vehicle (Ora syrup and Ora Plus suspending vehicle; Paddock Laboratories Inc., Minneapolis, MN).

Experimental Design

The rabbits were anesthetized by intraperitoneal administration of xylazine (6.6 mg/kg of body weight) and ketamine (100 mg/kg of body weight). The eyes were scarified in a 2 × 2 cross-hatch pattern and each one was inoculated with 5×10^5 PFU of virus. All corneas developed dendrite lesions as verified by slit lamp examination. The rabbits were visually examined daily for health and eye conditions for 27 days. The eyes were examined with a slit lamp on PI day 27 to verify the absence of ocular abnormalities; none were found. Tears were collected on nylon swabs from the rabbit eyes for 6 days before treatment (PI days 28–33) was initiated. Tear samples were processed for DNA extraction followed by real-time PCR for HSV-1 DNA quantification. The rabbits were divided into five statistically balanced groups based on the mean HSV-1 DNA copy number, number of positive shedding days, and number of positive swabs. Group 1 received no treatment; group 2 received vehicle orally twice daily; group 3 received 7 mg/kg; group 4 received 70 mg/kg; and Group 5 received 140 mg/kg VCV orally twice daily. Treatment was given at 12-hour intervals (7 AM and 7 PM) for 14 days from PI days 36 to 49. Tear swabs were collected from all rabbit eyes once a day (6 hours after first treatment) from PI days 40 to 49. The swabs were stored at −20°C until the DNA was extracted.

DNA Elution

DNA elution was performed according to the previously described method.[7] The DNA was eluted with a DNA elution kit (Gentra Puregene; Qiagen Sciences, Germantown, MD), according to the manufacturer's instructions. The DNA samples were stored in DNA hydration buffer (provided with the kit) at 4°C until processed by real-time PCR. Sterile, unused swabs were processed as a negative control, and other swabs spiked with HSV-1 (McKrae strain) were processed as a positive control for DNA extraction by the same method. Swabs from naïve rabbit eyes were used as an additional negative control.

HSV-1 DNA Quantification

The HSV-1 copy number from the DNA samples was determined by calculating the number of DNA polymerase genes in the sample according to a published method.[7] The sequences of forward and reverse primers were 5′-AGA GGG ACA TCC AGG ACT TTG T-3′ and 5′-CAG GCG CTT GTT GGGT GTA C-3′, respectively (Integrated DNA Technologies [IDT], Coralville, IA). The primer pair was synthesized by IDT. The probe was 5′6-FAM/ACC GCC GAA CTG AGC A/3′ BHQ-1 (IDT). All reactions were performed in a total volume of 20 μL. The 20 μL of reaction mixture contained 1× PCR master mix (TaqMan Universal Mastermix; Applied Biosystems, Inc. [ABI], Foster City, CA), 100 nM of primers and probe, and 5 μL of DNA sample. All reactions were performed in 96-well plates (Bio-Rad, Hercules, CA), which were centrifuged for ~1 minute at 1000*g* at room temperature in a swinging-bucket rotor (CRU 5000 centrifuge; Damon/IEC, Needham, MA) to remove any air bubbles. The reaction conditions were as follows: 95°C for denaturation for 10 seconds, 55°C for annealing for 30 seconds, and 72°C extension for 10 seconds in a real-time PCR system (iCycler iQ; Bio-Rad) for 45 cycle repeats. All samples were analyzed in triplicate. Each reaction plate contained both the positive and negative controls. The cosmid containing the HSV-1 DNA polymerase gene was obtained from David Bloom (University of Florida, Gainesville, FL) and used as a standard. The cosmid contained a copy of the 4.8-kb restriction fragment (*Hin*dIIIA) encompassing the HSV-1 DNA polymerase gene from the HSV-1 strain 17Syn+. A standard curve was generated from 10- and 2-fold serial dilutions of the p*Hin*dIIIA cosmid.

Statistical Analysis

Standard statistical procedures were used to determine the mean and SEM. Detailed statistics were used to determine the *P*-values among the five treatment groups for the HSV-1 DNA copy number and the total positives per total swabs. Longitudinal series of observations were analyzed to include intersubject correlation. The data were analyzed by repeated-measures analysis of variance (ANOVA). Our main interest was in the significance of treatments, a between-subject effect. *P*-values given are those for the *F* test of the repeated-measures ANOVAs.

RESULTS

The HSV-1 DNA copy number was determined for 25 rabbits, which were divided into five groups according to the number positive for disease, number of positive eye swabs, and mean copy number (Table 1). All rabbits in all groups were positive at least once for HSV-1 DNA. The HSV-1 DNA mean copy number in the groups before treatment are group 1, 370 ± 70; group 2, 569 ± 237; group 3, 368 ± 86; group 4, 408 ± 108; and group 5, 396 ± 91. The percentage of positive swabs per total swabs before treatment in the five groups were group 1, 19%; group 2, 31%; group 3, 25%; group 4, 25%; and group 5, 33% (Table 1).

Effect of Treatments

The mean copy number in the untreated, placebo, 7-mg/kg VCV, 70-mg/kg VCV, and 140-mg/kg VCV groups was 232 ± 183, 564 ± 186, 518 ± 122, 67 ± 63, and 13 ± 7, respectively (Table 2, Fig. 1). The 70- and 140-mg/kg groups had significantly lower copy numbers of HSV-1 DNA shed into tears ($P < 0.05$) and total positive swabs ($P < 0.05$). The 70- and 140-mg/kg treatment groups had only 7 positive swabs of 100 total swabs. The other three groups had 19 to 24 positive swabs (Table 2).

TABLE 2. Number of HSV-1–Positive Rabbits, Eyes, and Swabs and Mean Copy Number after Treatment, 40 to 49 Days PI

Groups	Positive Rabbits	Positive Eyes	Positive Swabs (%)	Mean Copy Number ± SEM
Untreated	4/5	5/10	19/100 (19)	232 ± 183
Placebo	4/5	5/10	24/100 (24)	564 ± 186
7 mg/kg VCV	4/5	5/10	20/100 (20)	518 ± 122
70 mg/kg VCV	2/5	2/10	7/100 (7)	67 ± 63
140 mg/kg VCV	1/5	2/10	7/100 (7)	13 ± 7

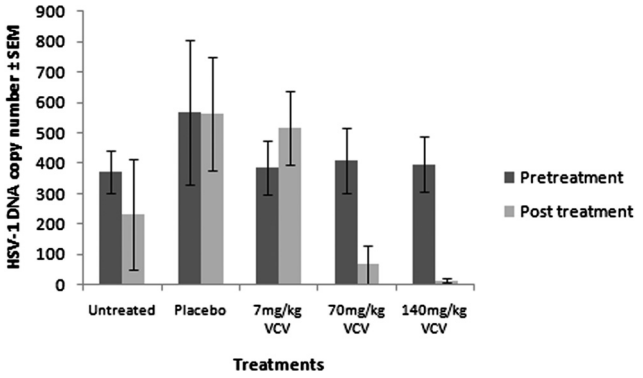

FIGURE 1. The HSV-1 mean copy number ± SEM in each treatment group is shown before and after treatment. Swabs were collected for 6 days (PI days 28–33) before treatment and for 10 days (PI days 41–50) after treatment began.

Safety and Tolerance

None of the rabbits in any group exhibited signs of adverse effects. The intake of food and water was equal in all groups. No rabbit lost weight. In fact, all five groups gained weight equally during the treatment period and for 1 month after treatment.

DISCUSSION

In our study in 45 healthy humans, we were unable to reduce the HSV-1 DNA shedding in tears and saliva by using 500 mg VCV, either alone or in combination with aspirin.[7] In the present study, we tested two significantly higher concentrations of VCV to determine the effects on HSV-1 DNA shedding in latently infected rabbits. We found a significant decrease in the HSV-1 DNA shedding in tears in rabbits treated with both 70 and 140 mg/kg VCV (Fig. 2, percent positive swabs). A 7-mg/kg dose, which is similar to a 500-mg human dose, did not suppress HSV-1 DNA shedding in latently infected rabbits.

In another study, the VCV dose was selected based on a published report[9] and the U.S. Pharmacopoeia at the time the study started, stating that the effective minimum dose is 500 mg per day. In a study of 69 immunocompetent participants with genital HSV-2, a significant reduction, but not complete suppression, of HSV-2 DNA shedding was found with 500 mg VCV given twice daily.[12] Similarly, a once-daily dose of 500 mg VCV significantly reduced the risk of transmission of genital herpes.[9] Other studies using higher doses of VCV have shown reductions in HSV-2 DNA shedding, but none of these studies demonstrated complete suppression of shedding of viral DNA with VCV or any another antiviral.[12,13]

VCV, an oral prodrug of acyclovir (ACV), is converted to ACV by first-pass intestinal and/or hepatic metabolism, and at least 90% of the ACV is excreted in the urine. VCV is more efficiently absorbed, and serum concentrations are increased more rapidly than ACV; VCV is effective with less frequent administration.[16] In a clinical setting in which VCV, the L-valyl ester of acyclovir, was orally administered to humans, approximately 54% of the dose was absorbed. Of the absorbed VCV, more than 99% was rapidly converted to acyclovir to give high plasma acyclovir concentrations and low plasma VCV concentrations, which became undetectable at 3 hours after the dose was administered.[17] The peak concentration of acyclovir in serum was 27.1 ± 5.6 μM after three doses of 1 g VCV for 6 days.[18] The C_{max} and AUC are dose dependent and show no proportionality.[19,20] In one study, after single doses of VCV 100, 250, 500, and 750 mg, or 1 g given to eight healthy volunteers, the mean C_{max} (±SD) was 0.83 (0.14), 2.15 (0.50), 3.28 (0.83), 4.17 (1.14), and 5.65 (2.37) μg/mL, respectively; and the mean AUC (SD) was 2.28 (0.40), 5.76 (0.60), 11.59 (1.79), 14.11 (3.54), and 19.52 (6.04) h · μg/mL, respectively.[19,20] On the basis of these data, the C_{max} and AUC with a 10-g human dose (which is approximately equal to 140 mg/kg) would be 50.6 μg/mL and 186.01 h · μg/mL, respectively.

Although VCV is regarded as relatively safe, some reports suggest possible neuropsychiatric adverse effects in elderly subjects with neurologic disorders.[21] We observed no visible side effects, toxicity, or mortality in the rabbits treated with VCV doses as high as 140 mg/kg. All the rabbits were healthy, and no ocular abnormalities were observed in these high-dose–treated groups. The high-dose rabbits were observed for 1 month after treatment and had no adverse effects. The doses of 70 and 140 mg/kg are equivalent to approximately 5 and 10 g, respectively, in the average human. To the best of our knowledge, there are no toxicity data available for the 10-g VCV dose. Findings in studies with intraperitoneal 150 or 200 mg/kg/d VCV did not show any toxicity in rabbits and support our data with no adverse effects at a maximum dose of 140 mg/kg.[22,23] A dose of 2 g VCV four times a day (8 g/d) has been used for the treatment of cytomegalovirus (CMV) infection.[10] However, a potentially fatal thrombotic microangiopathy (TMA)-like syndrome has been reported in some immunocompromised patients receiving high-dose prophylactic VCV therapy (8 g/d) for CMV disease for prolonged periods.[10] The risk of this syndrome appears to be higher in patients with advanced HIV disease.[10] Although the clinical benefits of VCV in some immunocompromised patients may outweigh the risk of TMA, close monitoring for symptoms of TMA is indicated in all immunocompromised patients receiving high-dose VCV.[11]

Thus, a higher dose of VCV could be a better treatment to reduce asymptomatic shedding of HSV-1 DNA with the possibility of very limited or no adverse effects. The suppressive therapy could be important to reduce the transmission of HSV-1 DNA by asymptomatic shedding. VCV treatment is now being tested for suppression of HSV-1, HSV-2, Epstein-Barr virus, and CMV.[7,12,24,25] The suppressive therapy with VCV in HSV-2 subjects has also been shown to reduce the infectiousness of HIV-1 and slow HIV-1 disease progression.[26] We have shown reduction in HSV-1 DNA-positive swabs and HSV-1 DNA copy numbers by the use of 70 and 140 mg/kg of VCV (Table 2, Fig. 1) with treatment for 14 days. These data are important, because they confirm that high doses of VCV can significantly reduce asymptomatic

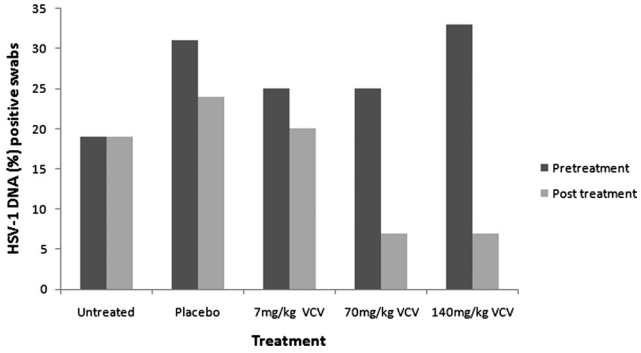

FIGURE 2. The number of positive swabs collected from PI days 41 to 50 for HSV-1 DNA in each treatment group are shown before and after treatment. Swabs were collected for 6 days (PI days 28–33) before treatment and for 10 days (PI days 41–50) after treatment began.

HSV-1 DNA shedding in an animal model with no observable adverse effects.

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